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14. ABSTRACT

The purpose of this study is to test whether mechanical stimulation of a co-cultured biomaterial scaffold can improve/expedite healing of a tendon-to-bone interface for soft tissue repair. There are several precursor milestones that need to be achieved before the purpose can be tested. Namely, these milestones include: determining an appropriate scaffold for the tendon to bone interface, characterizing the co-culture behavior on the selected scaffold, developing a mechanical bioreactor to stimulate co-cultured scaffolds, and characterize the effect of mechanical stimulation on the co-cultured scaffolds. The final test will be validated in a tendon-to-bone rabbit model. Currently, techniques have been established to condition the scaffolds by adsorbing attachments proteins found in fetal bovine serum. Efficient protocols for extracellular matrix digestion and analysis have been developed to save scaffold materials and correlate data. Finally, a customizable bioreactor was designed to selectively mechanically stimulate tendon-to-bone tissue engineering co-cultured scaffolds.

15. SUBJECT TERMS
Scaffold, Tissue Engineering, Bioreactor, Tendon, Bone, Biomaterial, Extracellular Matrix, Animal model

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INTRODUCTION

Tissue engineering techniques have been well established for single tissue types[1-6]. Interface tissue engineering is being researched more frequently[7,8]. One specific area of interface engineering is tendon-to-bone interfaces, called enthesis[9-12]. The purpose of this study is to test whether mechanical stimulation of a co-cultured biomaterial scaffold can improve/expedite healing of a tendon to bone interface for soft tissue repair. There are several precursor milestones that need to be achieved before the purpose can be tested. Namely, these milestones include: determining an appropriate scaffold for the tendon to bone interface, characterizing the co-culture behavior on the selected scaffold, developing a mechanical bioreactor to stimulate co-cultured scaffolds, and characterize the effect of mechanical stimulation on the co-cultured scaffolds. The final test will be validated in a tendon to bone animal model. These milestones are outlined and described in this report.

BODY

A forward on the organization of the section: body. The body will be organized based on the approved specific aims from the original grant. The specific aim will be repeated verbatim from the grant and then a brief methods description, followed by relevant data and discussion pertaining to the specific aim will be presented.

Specific Aim #1 : Preparation of the candidate scaffold materials, including non-degradable woven polyester fabric degradable woven PLA fabric, and biologic collagen scaffold for cell adhesion and proliferation

Task 1: Coat the scaffolding materials with fibronectin and/or collagen (months 0-2)

1.a coat with human fibronectin and/or collagen (Quarter 1, University of Memphis)

1.b confirm protein adsorption (Quarter 1, Antibody labeling of proteins, University of Memphis)

As communicated in the previous annual report, **Specific Aim 1 has been completed and a full discussion is included in the OCT 2011 Annual Report.** Briefly, the conclusions for specific aim 1 were that neither human fibronectin nor collagen coatings induced higher cell numbers than no coating. Second, soaking the scaffolds in fetal bovine serum prior to cell seeding is the preferred method to condition the scaffolds for cell culture and will be used for the remaining cell culture studies.

Specific Aim #2: Optimize the tissue-specific coatings using osteoblasts and fibroblasts in co-culture on the available scaffolding materials. Narrow the scaffolding materials to one selection

Task 2: ECM coating on scaffolds (months 1-6)

2.a. Seed osteoblasts on scaffolds for 7, 14, 21, and 28 days (Quarters 1-2, University of Memphis)

2.b. Seed fibroblasts on scaffolds for 7, 14, 21 and 28 days (Quarter 1-2, University of Memphis)

Task 2 was completed and a full discussion is included in the APR 2012 Annual update. Briefly, mechanical testing of scaffolds (not initially specified in the original grant), Live/Dead staining over 28 days and scanning electron microscopy (SEM) were performed. Mechanical testing determined that the PLA fabric is the best candidate based on degradability and good tensile properties. SEM showed cells attached and deposited matrix

on the scaffolds. Live/Dead staining showed good cell viability over the 28 day study for all scaffolds.

Task 3: Characterization of ECM coated scaffolds (months 3-6)

3.a. Cell attachment studies (Quarter 2, University of Memphis)

Task 3.a was completed and a full discussion is included in the APR 2012 Annual update. The FBS coated scaffolds had higher cell attachment than the fibronectin coated scaffolds. This data in conjunction with the live/dead fluorescent imaging shows that scaffolds coated with FBS provides a good attachment site for the cells.

Task 3.b. Cell proliferation studies (Quarter 2, University of Memphis)

Task 3.c. ECM composition studies total collagen and GAGs (Quarter 2, University of Memphis)

Task 3 was completed and a full discussion is included in the APR 2012 Annual update.

Task 4: Tissue selective ECM coating on scaffolds (months 6-9)

4.a. Seed osteoblasts and fibroblasts in co-culture for 7, 14, 21, and 28 days (Quarter 3, University of Memphis)

4.b. Characterization of Co-cultured scaffolds, repeat 3.a, 3.b, and 3.c. (Quarter 3, University of Memphis)

Task 4 was completed and a full discussion is included in the NOV 2012 Annual update. Briefly, a co-culture medium formulation consisting of alpha-MEM + 10% fetal

bovine serum (FBS) + 1x antibiotic/antimycotic + 25 ug/mL ascorbic acid + 3 mM beta glycerophosphate (β-GP) was determined for the fibroblasts and osteoblasts. A co-cultured cell tracking and migration study was performed using fluorescence labeling of the fibroblasts and osteoblasts demonstrating that the scaffolds are seeded to form separate and discrete "tendon" regions and "bone" regions. Finally, cells were seeded on the scaffolds in co-culture and the deposited extracellular matrix (ECM) was quantified for the 28 day study. We found cell viability was high and matrix deposition was continuous for the duration of the 28 days.

MILESTONE #1: Tissue Specific ECM coating on a scaffold (Month 9 - End of 3rd Quarter)

COMPLETED

Specific Aim #3: Enhance the optimized-tissue specific coating and scaffold by applying a cyclical tensile load during ECM deposition using a modified commercially available cell stretching system

Task 5: Mechanical stimulation of cell seeded scaffold (months 3-15)

5.a. Modify cell stretcher for chosen scaffolding material (Quarters 2-3, University of Memphis)

5.b. Seed cells on scaffold for 7, 14, 21, and 28 days with cyclic loading (Quarters 3-5, University of Memphis)

Task 5 was completed and a full discussion is included in the NOV 2012 Annual update. Briefly, a custom mechanical stimulating bioreactor was designed, built, and validated to allow dual straining of a single scaffold.

Task 6: Characterization of mechanically stimulated ECM coated scaffold (months 10-15)

6.a. Characterize scaffolds and ECM, repeat steps 3.a, 3.b, 3.c (Quarters 4-5, University of Memphis)

6.b *In vitro* Mechanical Tensile strength of ECM coated scaffolds (Quarter 5, University of Memphis)

Task 6 was completed and a full discussion is included in the NOV 2012 Annual update. Briefly, scaffolds were seeded in co-culture and mechanically stimulated in the bioreactor under different straining conditions (entirely strained or partially strained) to determine the effect of stimulation parameters. The deposited ECM was quantified. It was observed that the entirely strained stretching regime produced slightly higher amounts of ECM.

MILESTONE #2: Mechanically stimulated tissue specific ECM coated scaffold (month 15 End of 5th Quarter)

COMPLETED

Specific Aim #4: Evaluate the decellularized mechanically stimulated cytoselective tissue specific coating in a functional *in vivo* rabbit bone-tendon defect model.

The proof-of-principle in vitro study was performed as described in the April 2012 annual report. However, post operation complications with the surgery caused the study to be ended prematurely before relevant data could be acquired. Correspondence with IACUC and ACURO members was documented. The funding was not available to repeat the study,

so a PRORP approved alternate specific aim was performed. The revised specific aim is listed below.

Revised Specific Aim #4: Evaluate the decellularized mechanically stimulated cytoselective tissue specific coating using mesenchymal stem cells (MSCs) in culture

Task 10: Deposition of ECM on scaffolds and mechanical stimulation of scaffolds

10.a Seed fibroblasts and osteoblasts on the scaffold in co-culture and stimulate during culture in the mechanical bioreactor for 4 weeks. (Quarter 6, University of Memphis)

10.b After culture, scaffolds are decellularized using a free thaw and mild detergent solution. (Quarter 6, University of Memphis)

10.c Decellularized scaffolds are then seeded with rat MSCs for 24 hours (Quarter 6, University of Memphis)

Task 10 was completed and a full discussion is included in the NOV 2012 Annual update.

Task 11: Analyze MSC gene activation with rt-PCR.

11.a After culture on scaffolds for 24 hrs, cells are harvested and RNA is isolated. (Quarter 6, University of Memphis)

11.b Rt-PCR will be used to identify tissue specific markers for gene activation due to the ECM signaling of the tendon and bone side of the scaffold. (Quarter 6, University of Memphis)

Task 11 was completed and a full discussion is included in the NOV 2012 Annual update. Briefly, scaffolds were seeded in co-culture and mechanically stimulated in the bioreactor for 5 weeks. Afterwards scaffold were removed, decellularized, and re-seeded with rat mesenchymal stem cells (MSCs) for 24 hours to activate tissue specific genes. RT-PCR was used to measure gene activation. It was found that, generally the stretching had a larger impact on the osteoblast region compared to the fibroblast region. Stretching causes an increase in gene activation of the deposition of collagen, and a decrease in gene activation of mineralization.

Task 12: Analyze the ECM deposited on the scaffolds

12.a Each side of the scaffolds will analyzed for total collagen and GAG content (Quarter 6, University of Memphis)

12.b Histology will be performed on the ECM coated scaffolds to visually observe differences in the tendon-side and the bone side of the scaffolds. (Quarter 6, University of Alabama-Birmingham)

The ECM deposition data for Task 12.a is reported in Task 6, as these are duplicate tasks.

Histology was performed at the University of Alabama-Birmingham Center for Metabolic Bone Disease. After scaffolds had been cultured in the mechanical bioreactor and ECM had been deposited across the scaffolds, a half-stretched and all-stretched scaffold was removed from the chamber and frozen. Samples were then fixed in 10% Neutral Buffered Formalin for at least 24 hours, then transferred to 70% for complete fixation. Then all the samples were dehydrated through graded ethanols (80% ETOH X 1, 95% ETOH X 2, and 100% ETOH X 4) to three changes of xylene prior to the infiltration solution (95% Methyl Methacrylate,

(MMA), and 5% Dibutyl phthalate, (DBP). Infiltration solutions for all the samples were refreshed every 3 days, for a total of 4 changes. After infiltration, the samples were embedded on edge in a solution composed by 95% MMA and 5% DBP with 0.25% perkodox as the initiator. The samples were then exposed to UV light for polymerization. The fully polymerized (plasticized) sample blocks were trimmed (noting which end was the bone side) and cut to obtain 5um thin sections through the longitudinal axis. Methylene Blue & Basic Fuchsin (H&E like) stain, Goldner's Trichrome stain, Toluidine Blue stain, and Von Kossa stain were then performed. Figure 1 shows collage of histology slides from the four stains listed above for each of the three Half-stretched scaffold sections, the tendon region, the transition region, and the bone region. Figure 2 shows the same collage for the All-stretched scaffolds.

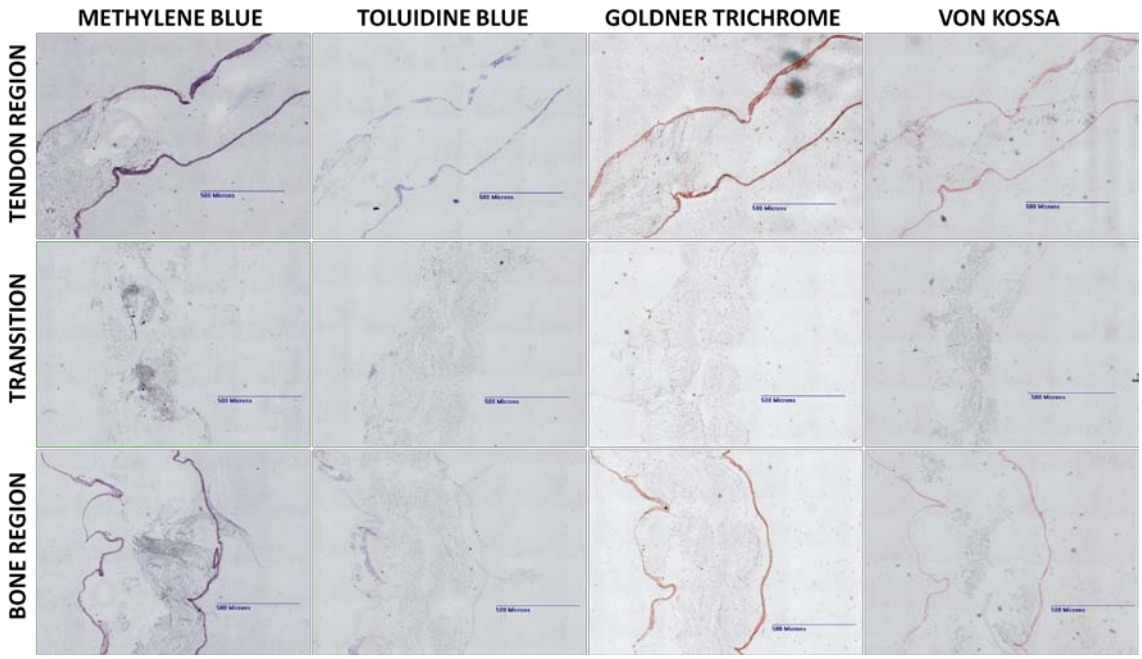

Figure 1 – Histological staining of mechanically-stimulated co-cultured scaffolds. These scaffolds are designated Half-stretched because the "tendon" side experienced strain while the bone side was held static. The four stains are listed in columns and the scaffold sections are listed in the rows. The fibroblast "tendon" region stained for the most ECM and little to no mineralization. The center transition was located under a clamp and no matrix was observed. The non-stretched osteoblast "bone" region stained less ECM and little to no mineralization.

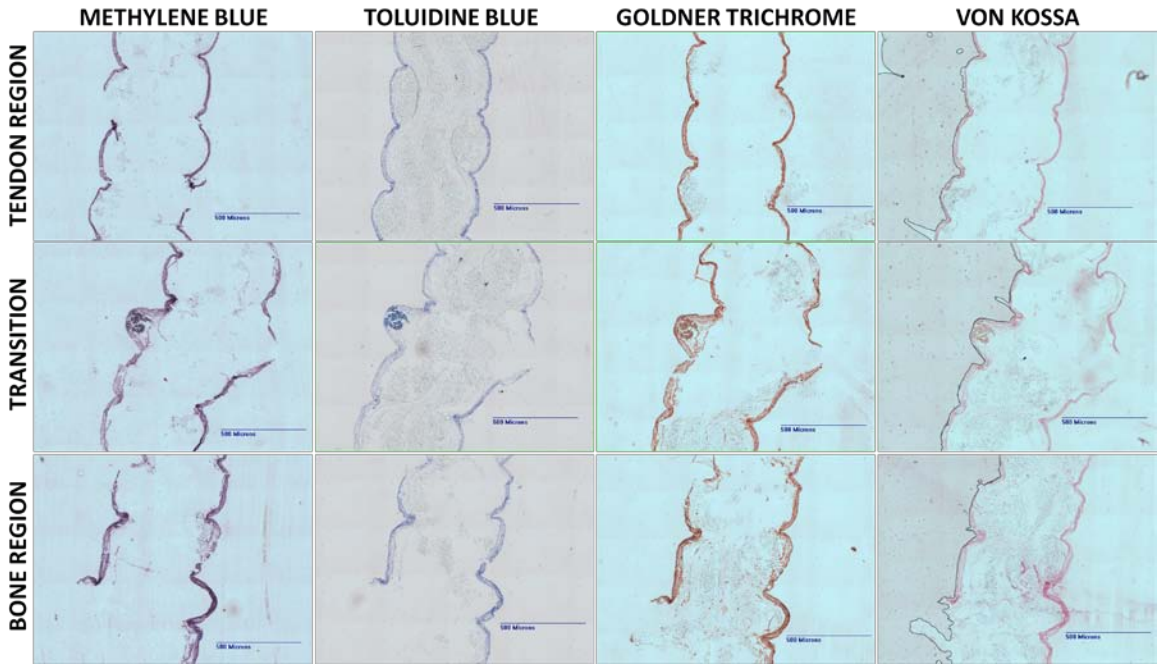

Figure 2 – Histological staining of mechanically-stimulated co-cultured scaffolds. These scaffolds are designated All-stretched because both the "tendon" side and the "bone" side experienced strain. The four stains are listed in columns and the scaffold sections are listed in the rows. ECM was uniformly deposited on the surface of the fabrics for each section (there was no center clamp obstructing ECM deposition as in the half stretched scaffolds). The dense cluster on the left side of the transition region is an indication thread used to mark the midpoint of the scaffold. Collagen was highly aligned over the entire scaffold in the direction of the tensile strain. There was little to no mineralization stain for any portion of the scaffold.

The half-stretched scaffolds in Figure 1 show that the stretched fibroblast "tendon" region had a thicker surface layer of deposited ECM than the static unstretched osteoblast "bone" region. This is most likely due to the mechanical strains acting on the fibroblasts, and not on the static osteoblasts, causing increased ECM deposition in the "tendon" region. The center transition area had no visible cells or matrix due to a clamp used in the bioreactor. The clamp is in place to allow dual strains of the scaffold (see NOV 2012 annual report Task 5). Cells were not able to survive between the clamps and therefore could not deposit ECM. When the center clamp is removed then both the fibroblast "tendon" side and the osteoblast "bone" side were exposed to cyclic strain. From Figure 2, the all-stretched ECM coating is more uniform and thicker in the transition and osteoblast regions. The cluster on the left

hand side of the transition images is a suture used to indicate the midpoint of the scaffold in order to keep track of the tendon and bone regions. Most all the stains used for both the Half-stretched and All-stretched scaffolds indicated high cell counts in multiple layers and highly oriented and aligned collagen fibers on the surface of the scaffolds. There was no observed mineralization in von kossa staining. Even though we did culture the scaffolds in an osteogenic medium containing β-GP, it was reduced to limit fibroblast calcification. So it is possible that the mineral was too diffuse to detect with histological stating. There was some cellular penetration into the weave and some small amount of ECM deposition near the surface. However, the vast majority of ECM deposition was limited to the scaffold surface. Figure 3 demonstrates a higher magnification view of the high cell numbers, highly aligned collagen, low fibrocartilage expression, and absent mineralization of the surface coating.

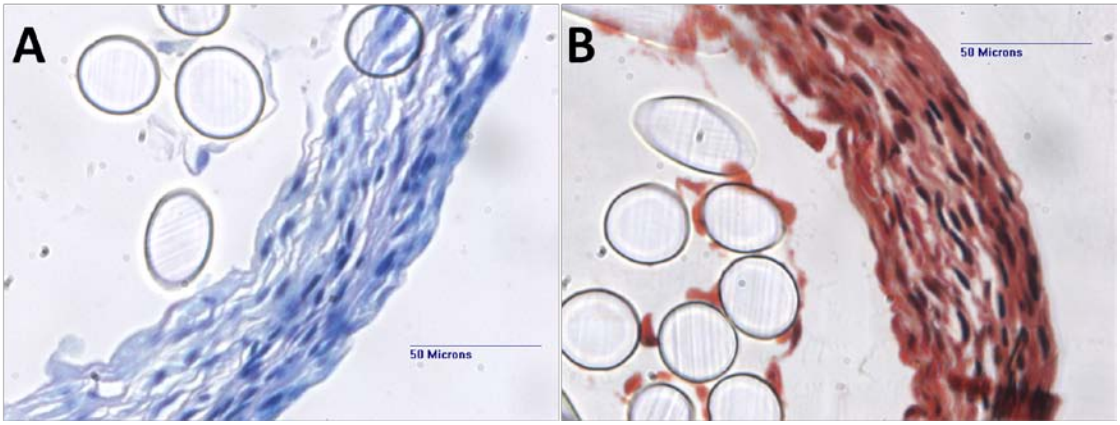

Figure 3 – Higher magnification (40x) of the collagenous surface coating. (A) Toluidine Blue stain of the ECM coating between the osteoblast and transition regions and (B) Goldner Trichrome stain of the ECM coating between the fibroblast and transition region. Both images shows multiple layers of highly aligned collagen, and high cell numbers. Image B also indicates a lack of fibrocartilage and mineral staining.

Task 12 has been completed.

MILESTONE #3: Report of animal data (month 18 end of end of 6th quarter)

KEY RESEARCH ACCOMPLISHMENTS

- Established scaffold conditioning protocols for cell attachment
- Established ECM digestion and analysis protocols that is applicable to multiple scaffold types
- Selected a single scaffold (PLA fabric) as an appropriate scaffold for tendon/bone interface
- Demonstrated separate co-cultured tissue specific regions on a single scaffold
- Built a custom bioreactor for selective mechanical stimulation in co-culture
- Histological testing exhibited a robust collagenous ECM coating on the scaffolds after mechanical conditioning in the custom bioreactor
- Demonstrated mechanically stimulated ECM activates tissue specific genes in stem cells

REPORTABLE OUTCOMES

This project is expected to have several reportable outcomes. The research will produce three or four manuscript submissions. These will include:

- Scaffold selection for tendon-to-bone tissue engineering (estimated submission date – 11 FEB 2013)
- Fabrication of a co-cultured tissue specific tendon-to-bone scaffold (estimated submission date – 22 FEB 2013)
- Development of a customizable bioreactor for adjustable scaffold stimulation (estimated submission date – MAR 2013)
- Evaluation of a tissue-specific scaffold for tendon-to-bone healing in a rabbit model (estimated submission date – 30 MAR 2013)

The following list of presentations was published from this grant:

- Cooper JO, Goodhart M, Bumgardner JD, Haggard WO. 2013 (accepted). Mechanically-Stimulated Co-cultured Tissue-Specific Scaffolds for Tendon/Bone Interface Engineering. *Society for Biomaterials* Annual Meeting. Boston, MA.

- Goodhart M, Cooper JO, Haggard WO, Bumgardner JD. 2012. Design and Validation of a Cyclic Strain Bioreactor to Condition Spatially-Selective Scaffolds in Dual Strain Regimes. *Biomedical Engineering Society*. Atlanta, GA.

- Cooper JO, Bumgardner JD, Williams JL, Cole JA, Smith RA, Haggard WO. 2012. Co-Cultured Tissue-Specific PLA Fabric Scaffolds for Tendon/Bone Interface Tissue Engineering. *Military Health System Research Symposium* Annual Meeting. Ft. Lauderdale, FL.

- Goodhart M, Cooper JO, Haggard WO, Bumgardner JD. 2012. Design and Validation of a Cyclic Strain Bioreactor to Condition Spatially-Selective Scaffolds in Dual Strain Regimes. *Military Health System Research Symposium* Annual Meeting. Ft. Lauderdale, FL.

- Cooper JO, Bumgardner JD, Williams JL, Cole JA, Smith RA, Haggard WO. 2012. **(1st Place Award for Research in Engineering)** Scaffold Selection for Tendon/Bone Tissue Engineering. *24th Annual University of Memphis, Student Research Forum*. Memphis, TN

This project will also support the fulfillment of two graduate degrees:

- Master's Thesis: Development of a mechanically stimulating bioreactor for tendon-bone tissue engineering (August 2012)
- PhD Dissertation: Tendon-bone tissue engineering (December 2012)

CONCLUSION

The work to this date has pointed out a clear pathway for tissue engineering methods for tendon-to-bone interfaces. Specifically, detailed cell culture techniques and protocols, including cell seeding, ECM digestion and analysis have been established. Based on mechanical testing and ECM deposition on the scaffolds, the best choice as a scaffolding material for this project is the commercially available PLA fabric manufactured by Synthasome, Inc. It was demonstrated that the PLA scaffold could support co-cultured fibroblasts and osteoblasts in separate tissue specific regions on a single scaffold to create a tendon region and bone region. Also a novel custom bioreactor was designed and built for the specific task of stimulation of a tendon directed (fibroblast) end of a scaffold and non-stimulation of a bone directed (osteoblast) end of a scaffold with differing stimulation regimes. Through histological testing a robust collagenous ECM coating was uniformly applied over the entire scaffold. Finally, that deposited ECM coating on the scaffolds was shown to increase activation of tissue specific genes in rat mesenchymal stem cells compared to tissue culture plastic.

Future Work includes understanding and experimenting with the mechanical stimulation of the bioreactor. During our testing, we chose testing parameters (such as, % strain, strain duration, number of cycles, lag between cycles, etc.) that were commonly reported in the literature. There are many opportunities to tune our custom designed bioreactor to make a better tendon to bone scaffold. It would also be beneficial to test the deposited ECM for more specific matrix components. We tested for broad general indicators of cell behavior and ECM deposition, namely DNA, GAGs, and collagen, to understand the big picture of co-culture and mechanical stimulation of seeded scaffolds. It would be advantageous to fill in the picture with details of tissue specific collagens like, type I, II, III, V, and X plus other

tissue specific markers like decorin, scleraxis, tenacin-C, aggrecan, COMP, osteopointin, osteocalcin and alkaline phosphatase to name a few. Lastly, evaluating the mechanically-conditioned co-cultured scaffolds in an *in vivo* animal model would also help develop this technology through observing tissue integration of the tendon and bone regions into the targeted tissues. Even though we referenced peer-reviewed and published animals in our *in vivo* attempt, we were not able to anticipate post operative complications. In the next attempt a pilot study of a few animals will help sort out the complications in the complex tendon-to-bone healing model. Overall, there is promising evidence that this technology would help tendon-to-bone interface repair.

REFERENCES

1. Guo C, Spector M. Tissue Engineering of Tendons and Ligaments. In: Ma PX, Elisseeff J, editors. Scaffolding in Tissue Engineering. Boca Raton: CRC Press; 2005. p 385-411.
2. Louie L, Yannas I, Spector M. Tissue Engineered Tendon. In: Patrick C, Mikos A, McIntire L, editors. Frontiers in Tissue Engineering. New York: Pergamon; 1998. p 412-442.
3. Caterson E, Tuan R, Bruder S. Cell Based Approaches to Orthopedic Tissue Engineering. In: Goldberg V, Caplan A, editors. Orthopedic Tissue Engineering: Basic Science and Design. New York: Marcel Dekker, Inc.; 2004. p 21-50.
4. Dourte LM, Kuntz AF, Soslowsky LJ. Twenty-five years of tendon and ligament research. J Orthop Res 2008;26(10):1297-305.
5. Goldberg V, Caplan A. Princlples of Tissue Engineering and Regeneration of Skeletal Tissues. In: Goldberg V, Caplan A, editors. Orthopedic Tissue Engineering. New York: Marcel Dekker; 2004. p 1-10.
6. Valentin JE, Badylak JS, McCabe GP, Badylak SF. Extracellular matrix bioscaffolds for orthopaedic applications. A comparative histologic study. J Bone Joint Surg Am 2006;88(12):2673-86.
7. Dormer NH, Berkland CJ, Detamore MS. Emerging techniques in stratified designs and continuous gradients for tissue engineering of interfaces. Ann Biomed Eng;38(6):2121-41.
8. Wang IE, Shan J, Choi R, Oh S, Kepler CK, Chen FH, Lu HH. Role of osteoblast-fibroblast interactions in the formation of the ligament-to-bone interface. J Orthop Res 2007;25(12):1609-20.
9. Benjamin M, Milz S, Bydder GM. Magnetic resonance imaging of entheses. Part 1. Clin Radiol 2008;63(6):691-703.
10. Derwin KA, Baker AR, Spragg RK, Leigh DR, Iannotti JP. Commercial extracellular matrix scaffolds for rotator cuff tendon repair. Biomechanical, biochemical, and cellular properties. J Bone Joint Surg Am 2006;88(12):2665-72.

11. Cooper JA, Lu HH, Ko FK, Freeman JW, Laurencin CT. Fiber-based tissue-engineered scaffold for ligament replacement: design considerations and in vitro evaluation. Biomaterials 2005;26(13):1523-32.
12. Spalazzi JP, Dagher E, Doty SB, Guo XE, Rodeo SA, Lu HH. In vivo evaluation of a tri-phasic composite scaffold for anterior cruciate ligament-to-bone integration. Conf Proc IEEE Eng Med Biol Soc 2006;1:525-8.
13. Singh P, Carraher C, Schwarzbauer JE. Assembly of Fibronectin Extracellular Matrix. Annual Review of Cell and Developmental Biology, Vol 26 2010;26:397-419.
14. Kim SH, Turnbull J, Guimond S. Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. Journal of Endocrinology 2011;209(2):139-151.
15. Shoulders MD, Raines RT. Collagen Structure and Stability. Annual Review of Biochemistry 2009;78:929-958.
16. Schor SL. Cell proliferation and migration on collagen substrata in vitro. J Cell Sci 1980;41:159-75.
17. Sawyer AA, Hennessy KM, Bellis SL. The effect of adsorbed serum proteins, RGD and proteoglycan-binding peptides on the adhesion of mesenchymal stem cells to hydroxyapatite. Biomaterials 2007;28(3):383-92.
18. Johnson GA, Tramaglini DM, Levine RE, Ohno K, Choi NY, Woo SL. Tensile and viscoelastic properties of human patellar tendon. J Orthop Res 1994;12(6):796-803.
19. Buckley MJ, Banes AJ, Levin LG, Sumpio BE, Sato M, Jordan R, Gilbert J, Link GW, Tran Son Tay R. Osteoblasts increase their rate of division and align in response to cyclic, mechanical tension in vitro. Bone Miner 1988;4(3):225-36.
20. Androjna C, Spragg RK, Derwin KA. Mechanical conditioning of cell-seeded small intestine submucosa: a potential tissue-engineering strategy for tendon repair. Tissue Eng 2007;13(2):233-43.
21. Abousleiman RI, Reyes Y, McFetridge P, Sikavitsas V. Tendon tissue engineering using cell-seeded umbilical veins cultured in a mechanical stimulator. Tissue Eng Part A 2009;15(4):787-95.
22. Butler DL, Hunter SA, Chokalingam K, Cordray MJ, Shearn J, Juncosa-Melvin N, Nirmalanandhan S, Jain A. Using functional tissue engineering and bioreactors to mechanically stimulate tissue-engineered constructs. Tissue Eng Part A 2009;15(4):741-9.
23. Garvin J, Qi J, Maloney M, Banes AJ. Novel system for engineering bioartificial tendons and application of mechanical load. Tissue Eng 2003;9(5):967-79.
24. Riboh J, Chong AK, Pham H, Longaker M, Jacobs C, Chang J. Optimization of flexor tendon tissue engineering with a cyclic strain bioreactor. J Hand Surg Am 2008;33(8):1388-96.
25. Hashimoto Y, Yoshida G, Toyoda H, Takaoka K. Generation of tendon-to-bone interface "enthesis" with use of recombinant BMP-2 in a rabbit model. J Orthop Res 2007;25(11):1415-24.